

## WHAT IS CLAIMED IS:

1. A method of interrogating a field having a plurality of PREs distributed therein, comprising illuminating the field with an optical light source,  
 5 detecting a spectral emission characteristic of individual PREs and other light scattering entities in the field,  
 constructing a computer image of the positions and values of the emission spectral characteristic of individual PREs and other light-scattering entities present in the field, and  
 discriminating PREs with a selected spectral signature from other light-scattering entities based  
 10 on detected spectral characteristic values unique to the selected-signature PREs, to provide information about the field.
2. The method of claim 1, wherein said detecting includes simultaneously detecting the spectral emission characteristic of the light-scattering entities in the field.
3. The method of claim 2, wherein said detecting further includes detecting the spectral emission characteristic of the light scattering entities in the field simultaneously at a plurality of defined spectral frequencies.
4. The method of claim 1, wherein said illuminating and detecting steps include  
 20 illuminating said PREs with incident light predominantly in a first frequency band;  
 detecting the spectral emission characteristics of individual PREs and other light scattering entities in the field under illumination at the first frequency band;  
 illuminating said PREs with incident light  
 25 predominantly in a second frequency band; and  
 detecting the spectral emission characteristics of individual PREs and other light scattering entities in the field under illumination at the second frequency band.
5. The method of claim 1, wherein said detecting includes sequentially detecting the spectral emission characteristic of individual PREs and other light scattering entities in the field at a plurality of defined spectral bands.

6. The method of claim 1, wherein said illuminating includes exposing the field to a plurality of narrowband pulses of light which vary in duration, and said detecting includes detecting variations in emitted light intensity produced by variations in duration.

5 7. The method of claim 1, wherein at least some of the PREs are non spherical, said illuminating includes exposing the field to polarized light at different orientations and/or different angles of incident, and said discriminating includes detecting changes in a spectral emission characteristic as a function of incident light polarization orientation or angle.

10 8. The method of claim 1, wherein said PREs are formed in the field by a step selected from the group consisting of

(i) binding nucleation centers to a field, metal enhancing said nucleation centers, observing enhancement of said nucleation center during said metal enhancing process, and terminating enhancement when a PRE of a desired spectral characteristic has been formed;

15 (ii) adding pre-formed PREs to a target in the field,

(iii) making PREs at target sites in the field.

20 9. The method of claim 1, wherein discriminating PREs with a selected spectral signature from other light-scattering entities in the field includes discriminating a selected type of PRE from all other light-scattering entities in the field, based on detected values, for each light-scattering entity in the field, of peak position, peak intensity, or peak width at half intensity of the spectral emission curve, peak halfwidth in the image plane, and polarization or angle of incidence response.

25 10. The method of claim 9, wherein said discriminating is effective to discriminate, for a selected type of PREs, those selected PREs which are interacting with one another and those which are not.

30 11. The method of claim 9, wherein said discriminating is effective to discriminate a selected type of PRE from another selected type of PRE in the field.

35 12. The method of claim 1, wherein the PREs have surface-localized fluorescent molecules or Raman-active molecular entities, and said detecting includes detecting plasmon-resonance induced fluorescent emission or Raman spectroscopy emission from one or more of said molecules or entities, respectively.

13. The method of claim 1, for use in determining the total number of PREs of a selected type in a field, wherein said discriminating includes counting the number of PREs having a selected range of values of a selected spectral emission characteristic in the constructed computer image.

5 14. The method of claim 1, for use in determining a spatial pattern of PREs having a selected range of values of a selected spectral characteristic in the field, wherein discriminating includes constructing an image of the relative locations of PREs with those spectral-characteristic values.

10 15. The method of claim 14, wherein the location between two adjacent PREs is less than the Rayleigh resolution distance, and said detecting includes exposing the field with light of one wavelength, to obtain a diffraction image of PREs in the field, exposing the field with light of a second wavelength to obtain a second diffraction image of PREs in the field, and comparing the distance between peaks in the two diffraction patterns.

15 16. The method of claim 1, for use in interrogating a change in the environment of the field, wherein said discriminating includes comparing the values of the detected spectral characteristic of a PRE in the field before and after said change.

20 17. The method of claim 16, wherein the field is interrogated for changes in the dielectric constant of environment.

25 18. The method of claim 1, for use in detecting motion of PREs in the field, wherein said detecting includes detecting the centers of the diffraction patterns of the PREs in the image plane, as a function of time.

30 19. Apparatus ~~for use in the method of claim 1~~, for interrogating a field having a plurality of PREs distributed therein, comprising

an optical light source for illuminating the field,

an optical detector for detecting a spectral emission characteristics of individual PREs and other  
30 light scattering entities in the field, when the field is illuminated by the light source,

an image processor operatively connected to the detector for constructing, from signals received from the detector, a computer image of the positions and values of the spectral emission characteristic of individual PREs and such other light-scattering entities present in the field,

discriminator means for discriminating PREs with a selected spectral signature from other light-  
35 scattering entities in the computer image, and

output means for displaying information about the field based on the information about the selected PREs.

20. The apparatus of claim 19, wherein said light source includes a bright field/dark field lens  
5 for directing light onto the field.

21. The apparatus of claim 19, wherein said light source includes means for illuminating the field at each of a plurality of different wavelengths.

10 22. The apparatus of claim 19, wherein said detector is a two-dimensional photodetector array capable of detecting a spectral emission characteristic simultaneously from a plurality of illuminated PREs in an illuminated field.

15 23. The apparatus of claim 19, wherein said detector includes means for spectrally separating light emitted from the PREs, and said image processor operates to form a computer image of the positions and values of the emission spectral characteristic of individual PREs and such other light-scattering entities present in the field at each of a plurality of different emission wavelengths.

20 24. The apparatus of claim 23, wherein the optical detector includes a two-dimensional array of optical fibers whose output is aligned so as to constitute a line source that is sent into a grating or prism for responding to that line source, and a two-dimensional detector array for responding to the spread of spectral light of each fiber in said line source of detected light.

25 25. The apparatus of claim 19 or 23, which further includes means for moving said target in an x-y plane, relative to said light source, to successively illuminate individual light-scattering entities in the field.

30 26. The apparatus of claim 19, wherein said image processor operates to construct an image of PRE positions and, for each light-scattering entity in the field, values of a spectral characteristic selected from the group consisting of peak position, peak intensity, or peak width at half intensity of the spectral emission curve, peak halfwidth in the image plane, and polarization or angle of incidence response.

27. The apparatus of claim 19, wherein said image processor operates to construct an image of PRE positions and, for each light scattering entity in the field, a value of a spectral characteristic selected from the group consisting of fluorescence emission spectrum and Raman spectrum.

28. The apparatus of claim 19, wherein said discriminator means includes means for discriminating PREs with a selected spectral signature from all other light-scattering entities in the field, based on detected values, for each light-scattering entity in the field, of peak position, peak intensity, or peak width at half intensity of the spectral emission curve, peak halfwidth in the image plane, and polarization or angle of incidence response.

29. The apparatus of claim 29, wherein said discriminating is effective to discriminate for a selected type of PREs, those selected PREs which are interacting with one another and those which are not, or one selected type of PRE from another selected type of PRE in the field.

30. A composition of plasmon resonant particles (PRPs) having one or more populations of PRPs, and characterized by:

- (a) the PRPs have a width at halfheight of less than 100 nm;
- (b) the PRPs in a single population are all within 40 nm of a defined wavelength;
- (c) at least 80% of the PRPs in the composition satisfying criterion (a) are in one or more of the populations and have a spectral emission wavelength in a single range selected from the group consisting of:
  - (i) > 700 nm;
  - (ii) 400-700 nm; and
  - (iii) < 400 nm; and
- (d) each population has at most a 30% overlap in number of PRPs with any other population in the composition.

31. The composition of claim 30, wherein at least 80% of the PRPs in the composition are in one or more of the populations and have a spectral emission wavelength in the 400-700 nm wavelength range.

32. The composition of claim 30 or 31, wherein the particles have a composition selected from the group consisting of

- (i) a solid silver particle,
- (ii) a silver particle with a gold core, and

(iii) a particle with a dielectric core and an outer silver shell of at least about 5nm.

33. The composition of claim 30, wherein the particles have localized at their surfaces, one from the following group: (i) surface-attached ligands adapted to bind to ligand-binding sites on a target, where the ligand/ligand-binding sites are conjugate binding pairs, (ii) fluorescent molecules, (iii) Raman-active molecular entities, and (iv) a blocking reagent to prevent non-specific binding, and (v) a coating with functional groups for covalent coupling to the PRPs.

34. The composition of claim 33, wherein the surface localized ligand is one of a conjugate pair selected from the group of pairs consisting of antigen/antibody, hormone/receptor, drug/receptor, effector/receptor, enzyme/substrate, lipid/lipid binding agent and complementary nucleic acids strands.

35. The composition of claims 33, which includes first and second populations of PRPs having first and second different surface localized molecules or entities.

36. The composition of claim 35, for use in identifying a target having first and second ligand-binding sites, wherein the first and second surface bound molecules are first and second ligands effective to bind to said first and second ligand-binding sites, respectively.

37. The composition of claim 36, wherein the first and second surface-localized molecules are oligonucleotides having sequences that are complementary to first and second proximate sequence regions of a target polynucleotide.

38. The composition of claim 35, wherein the first and second surface-localized entities are Raman-active molecular entities with different Raman spectral characteristics.

39. The composition of claim 30, having first and second populations of PRPs, each with a different shape, at least one of which is spherical or tetrahedral.

40. A diagnostic method for use in detecting the presence of, or information about, a target having a molecular feature of interest, comprising  
contacting the target with one or more PRPs having surface localized molecules, to produce an interaction between the molecular feature and the localized molecules,  
illuminating the target with an optical light source, and

determining the presence of or information about the target by detecting a plasmon resonance spectral emission characteristic of one or more PREs after such interaction with the target.

41. The method of claim 40, wherein said target contains a ligand-binding site, the surface-localized molecule is one of a ligand/ligand-binding site conjugate pair selected from the group of pairs consisting of antigen/antibody, hormone/receptor, drug/receptor, effector/receptor, enzyme/substrate, lipid/lipid binding agent and complementary nucleic acids strands, said contacting produces a PRE/target complex, and said detecting includes detecting a plasmon resonance spectral emission characteristic of the complex.

42. The method of claim 41, wherein said contacting further includes the step of washing the field to remove PREs not bound to the target through a ligand/ligand-binding interaction.

43. The method of claim 41, wherein the target has at least two proximately spaced ligand-binding sites, and said complex includes at least two proximately spaced PREs that have a spectral emission signature different from that of PREs in the absence of binding to the target.

44. The method of claim 43, for determining the presence of a target having first and second proximately spaced ligand-binding sites, wherein said contacting includes reacting the target with first and second populations of PREs having surface-localized first and second ligands, respectively, for binding to the first and second ligand binding sites, respectively.

45. The method of claim 44, wherein the target is a polynucleotide having first and second adjacent base sequence regions, the ligand molecules on the first and second PREs are complementary to said first and second regions, and said contacting is carried out under conditions which allow surface-attached ligand molecules to hybridize with complementary-sequence regions of the target.

46. The method of claim 41, wherein the PRE(s) contain surface-localized fluorescent reporter molecules, and the interaction of a PRE with the target or with another PRE at the target is effective to detectably alter a plasmon-resonance induced spectral emission characteristic of the fluorescent molecules on the PRE.

47. The method of claim 41, wherein the PRE(s) contain surface-localized Raman reporter molecular entities, and the interaction of a PRE with the target or with another PRE at the target is

effective to detectably alter a plasmon-resonance induced spectral emission characteristic of the Raman entities on the PRE.

48. The method of claim 41, wherein the target has multiple ligand-binding sites, the PREs  
5 bind to two or more of these sites and said detecting includes constructing a spatial map of the sites of PRE attachment to the target, which is indicative of the relative spacings of the ligand-binding sites in the target.

49. The method of claim 48, for use in mapping regions of known sequence in a target  
10 polynucleotide which is in a substantially extended condition, wherein the target is contacted with a plurality of PREs, each having different surface-attached oligonucleotides effective to hybridize to one of the know-sequence regions of the target, said contacting is carried out under conditions which allow the PRE's surface-attached oligonucleotides to hybridize with the target's selected base sequences, and said detecting includes (i) washing the field to remove unbound PREs, and (ii) mapping the relative  
15 positions of the bound PREs according to their spectral emission characteristics.

50. The method of claim 40, for resolving the distance between two closely spaced target sites, wherein said PREs have substantially the same peak wavelength, wherein said detecting includes detecting a composite spectral emission characteristic of the two PREs including shifts and broadening  
20 of single-particle spectral peaks and appearance of new peaks.

51. The method of claim 40, for resolving the distance between two closely spaced target sites, wherein said PREs have different peak wavelengths, wherein said detecting includes separately detecting the center of the diffraction peak of each particles at different illuminating light wavelengths.  
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52. The method of claim 41, wherein said target includes an array of different-sequence oligo- or polynucleotides, the array is contacted with one or more PREs having one or more surface-attached polynucleotides, said contacting is carried out under conditions which allow the PRE's surface-attached polynucleotides to hybridize with the target array oligo- or polynucleotides, and said detecting includes  
30 (i) washing the target to remove unbound PREs, and (ii) detecting a spectral emission characteristic of PREs at each region of the array.

53. The method of claim 41, wherein said target is a polynucleotide present as a separated band in an electrophoresis gel, said contacting is carried out by exposing the surface of the gel to PREs under  
35 hybridization conditions.



55. The method of claim 41, wherein the molecular feature of interest is a molecule which functions catalytically to break a bond between two atoms in a molecular chain, said PRE includes a pair of PREs linked by said chain, said contacting is carried out under conditions effective to cleave the molecular chain, and said detecting includes detecting the disappearance of linked PREs or the appearance of unlinked PREs.

56. The method of claim 41, for detecting the presence of a target polynucleotide sequence having first and second contiguous nucleotide sequences, said contacting includes adding to the target, under hybridization conditions, first and second PREs having surface-localized first and second oligonucleotide probes complementary to the first and second target sequences, respectively, and treating the resulting hybridization product with a ligase enzyme, and said detecting includes detecting the presence of linked PREs.